

Influence of Different Carbon Sources on Exopolysaccharide Production by *Lactobacillus delbrueckii* Subsp. *bulgaricus* (B3, G12) and *Streptococcus thermophilus* (W22)

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ABSTRACT

Exopolysaccharides (EPSs) production was studied by Lactobacillus delbrueckii subsp. bulgaricus (B3, G12) and Streptococcus thermophilus (W22) in the medium containing various carbon sources (glucose, fructose, sucrose or lactose). For all the strains, glucose was the most efficient carbon source and B3, G12 and W22 strains produced 211, 175 and 120 EPS mg/L respectively. Also, the influence of different concentrations of glucose (5,10,15,20,25,30 g/L) on EPS production and growth was studied. The results indicated that EPS production and growth were stimulated by the high glucose concentration (30 g/L).

Key words: *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, exopolysaccharide production, carbon sources

INTRODUCTION

Several bacteria are known to synthesize the exopolysaccharides (EPSs). EPSs occur in two forms depending on their location: as a capsule (capsular polysaccharides) where the polymer is closely associated with the cell surface and as the slime polysaccharides that are loosely associated with the cell surface. Such a distinction may be difficult since some strains release capsular polysaccharidic material at the periphery (Tallon et al., 2003). For the cells, EPSs are thought to play a role in protection against the desiccation, toxic compounds, bacteriophages, osmotic stress, and to permit the adhesion to the solid surfaces and biofilm formation (De Vuyst and Degeest, 1999). In the food industry, these polymers are used as biothickeners because of their stabilizing, emulsifying or gelling properties. The bacterial

polysaccharides can also be differentiated by their chemical composition. The homopolysaccharides are composed of only one monosaccharide (glucose or fructose, mainly), and heteropolysaccharides are composed of at least two different monosaccharides. Other residues such as sn-glycerol- 3-phosphate, *N*- acetyl-aminosugars, phosphate, and acetyl groups can also be found (Laws et al., 2001).

Some lactic acid bacteria (LAB) secrete a polysaccharide polymer. This exopolysaccharide (EPS) is economically important because it can impart functional effects to foods and confer beneficial health effects (Welman and Maddox, 2003). Both *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* are known to produce heteropolysaccharides (De Vuyst et al., 2003). EPS characteristics and amounts can be influenced by several factors such

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as composition of the medium (carbon and nitrogen sources), as well as incubation conditions (temperature, pH, time, etc.) (Looijesteijn et al., 1999; De Vuyst and Degeest, 1999; Tallon et al., 2003). That explains the diversity of the contradictory results on exopolysaccharide production. EPSs produced by lactic acid bacteria (LAB) are the subject of an increasing number of studies. LAB are food grade organisms, possessing the generally-recognized-as-safe (GRAS) status, and can produce EPSs that are potentially useful as safe additives to improve texture and viscosity of natural fermented milk products and to prevent syneresis. Moreover, it has been suggested that some EPSs produced by lactic acid bacteria may confer health benefits to the consumer (Tallon et al., 2003).

Nowadays, a lot of effort is put into the selection of new microbial strains and optimization of culture conditions to achieve higher yields of those EPSs already commercially successful. Furthermore, there is a considerable interest in finding new EPSs that are suitable for special applications, or that have potential industrial relevance, either by applying different culture conditions or by using novel bacterial strains (Looijesteijn et al., 2000). Several studies have reported that the yield of EPS produced by the lactic acid bacteria can be influenced by changing the medium composition (Van Den Berg et al., 1995; Gamar et al., 1997; Grobber et al., 1997). However, less information is available relating the effect of medium composition and concentration on the EPS production.

The purpose of this study was to determine the influence of various carbon sources (glucose, fructose, sucrose or lactose) and different concentrations of glucose (5, 10, 15, 20, 25, 30 g/L) on the growth and EPS production.

MATERIALS AND METHODS

Isolation, identification and growth condition

Two strains of *L. delbrueckii* subsp. *bulgaricus* (G12, B3) and one strain of *S. thermophilus* (W22) were used in this study. *L. delbrueckii* subsp. *bulgaricus* strains were inoculated in MRS broth (Oxoid), while *S. thermophilus* strain was inoculated in M17 broth (Oxoid). All the strains were stored at -80 °C in MRS/M17 broth with 10% glycerol, and regenerated twice before the use.

Gram stains and catalase tests were conducted to identify strains. Also, carbohydrate fermentation characteristics of all the strains were determined by using API 50 CHL identification system. Results were recorded after 48 h at 42°C. Finally, the isolates were identified by 16S rDNA sequence (Tilsala-Timisjarvi and Alatossava, 1993; Ventura et al., 2000; Roy et al., 2001). The sequences obtained were searched against The Gen Bank DNA database using the blast function.

Culture conditions for EPS production

L. delbrueckii subsp. *bulgaricus* B3, G12 and *S. thermophilus* W22 were grown at 42 °C for 18 h in MRS and M17 media, respectively. Modified MRS media, containing 20 g of glucose (MRS-glu), lactose (MRS-lac) or fructose (MRS-fru)/L, instead of the 20 g glucose/L normally present in MRS medium, were used to examine the EPS production by *L. delbrueckii* subsp. *bulgaricus* strains. Also, the modified M17 medium, containing 20 g of lactose (M17-lac), sucrose (M17-suc), fructose (M17-fru) or glucose (M17-glu)/L, instead of the 5 g lactose/L normally present in the M17 medium, were used to examine the EPS production by *S. thermophilus* strain. The yeast extract was taken out from the MRS and M17 media. All the media were autoclaved for 15 min at 121 °C. The sugars were autoclaved separately and added to the cooled basal media. To study the influence of the carbon source concentration on the EPS production by the strains B3, G12 and W22, glucose was added to MRS and M17 media at concentrations of 5, 10, 15, 20, 25, 30 g/L.

Isolation and quantification of EPS

After the inoculation, the cultures were incubated at 42 °C for 18 h and then were boiled at 100 °C for 10 min. After cooling, they were treated with 17% (v/v) of 85% trichloroacetic acid solution and centrifuged (Frengova et al., 2000). The removal of the cells and protein was done by centrifugation. The EPS was precipitated with the ethanol (100% v/v). It was recovered by centrifugation at 4 °C at 14000 rpm for 20 min. Total EPS (expressed as mg/L) was estimated in each sample by the phenol-sulphuric method (Dubois et al., 1956) using glucose as the standard (Torino et al., 2001).

Statistical analysis

The statistical analysis was performed by the SPSS (Version, 12.0). In media (MRS and M17, respectively) containing different concentrations of glucose, Person's correlation was used to determine any significant difference between the EPS production and optical density of the strains. The significance was determined at $\alpha = 0.01/0.05$ level. If the significant differences were obtained among the treatment means ($P \leq 0.01/0.05$), then the means were differentiated using the least square mean test at $\alpha \leq 0.01/0.05$.

RESULTS AND DISCUSSION

The influence of carbon sources (fructose, lactose or glucose for *L. delbrueckii* subsp. *bulgaricus*, sucrose, fructose, lactose or glucose for *S. thermophilus*) on the growth and production by *L. delbrueckii* subsp. *bulgaricus* B3, G12 strains and *S. thermophilus* W22 strains was studied. Using fructose, lactose and glucose the strain B3 produced 180, 203 and 211 mg/L EPS, respectively, which was by G12 produced was 69, 113 and 175 mg/L, respectively. When fructose was used as the carbon sources, the EPS production dramatically decreased. For *S. thermophilus* strain W22, with sucrose, fructose, lactose and glucose EPS 34, 40, 114 and 120 mg/L, respectively (Table 1).

Table 1- Influence of different carbon sources on extracellular polysaccharide (EPS) production

Strains	Carbon Source	OD ₆₀₀ ^a	EPS (mg/L) ^a
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i>			
G12	Fructose	1.99±0.00	69±4
	Lactose	1.57±0.00	113±2
	Glucose	1.76±0.03	175±0
B3	Fructose	1.94±0.00	180±0
	Lactose	1.96±0.02	203±0
	Glucose	1.98±0.00	211±1
<i>S. thermophilus</i>			
W22	Sucrose	2.01±0.00	34±4
	Fructose	2.04±0.04	40±0
	Lactose	2.05±0.04	114±0
	Glucose	2.66±0.01	120±0

^a: Values are the means ± standard deviations of triplicate measurements

The total yield of EPS produced by the lactic acid bacteria depends on the composition of the medium and conditions in which the organisms grow (i.e., medium, temperature and incubation time) (Cerning et al., 1990; Cerning et al., 1994). Similar observations have been made for gram-negative bacteria, including *Klebsiella* sp., *Acinetobacter calcoaceticus* (Bryan et al., 1986), and *Aeromonas salmonicida* (Bonnet et al., 1993). Present results showed that the yield of the EPS produced by *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* strains depended on the carbon source and concentration present in the medium. Glucose was the most efficient carbon source for the EPS production, whereas fructose (for *L.*

delbrueckii subsp. *bulgaricus*) and sucrose (for *S. thermophilus*) were not efficient carbon sources.

The type of carbon source has a huge influence on EPS productivity and may also affect the composition of EPS. *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 produce three times more EPS with glucose than with fructose as a sugar source and the type of EPS produced by this organism was influenced by the sugar source as well (Grobben et al., 1997). Grobben et al. (1996) noted that the regulation of the EPS biosynthetic pathway in *L. bulgaricus* NCFB 2772 could be dependent on the carbohydrate source. Cerning et al. (1994) reported that *L. casei* CG11 was able to produce the EPS in the presence of various sugars (glucose, galactose, lactose, sucrose, maltose,

melibiose); however, the most efficient carbon source was glucose.

When *L. delbrueckii* subsp. *bulgaricus* B3, G12, and *S. thermophilus* W22 were grown in media (MRS and M17, respectively) containing different concentrations of glucose (5,10,15,20,25,30 g/L), the EPS production increased considerably (Table 2). The EPS yield obtained with 5 g of glucose per

liter in *S. thermophilus* W22 was 32 mg/L, while the EPS yield obtained with 30 g/L glucose per liter in *L. delbrueckii* subsp. *bulgaricus* G12 was 255 mg/L. All the three strains produced considerably more EPS when grown in the media containing glucose at high concentration (30 g/L). Furthermore, there were significant differences in the growth (as measured by OD) (Table 2).

Table 2- Influence of different concentrations in media containing glucose on extracellular polysaccharide (EPS) production

Concentration (g/l)	OD ₆₀₀ ^a	EPS (mg/L) ^a
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> G12		
5	0.47±0.01	146±0
10	0.74±0.04	147±1
15	0.94±0.06	160±0
20	1.76±0.03	175±0
25	2.77±0.04	181±2
30	3.30±0.06	255±3
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> B3		
5	0.59±0.00	116±0
10	0.83±0.03	137±1
15	1.26±0.02	143±0
20	1.98±0.00	211±1
25	2.19±0.02	221±3
30	2.27±0.00	224±2
<i>S. thermophilus</i> W22		
5	1.08±0.04	32±1
10	1.24±0.03	55±0
15	1.99±0.00	65±0
20	2.66±0.01	120±0
25	2.99±0.00	138±3
30	3.09±0.04	174±4

^a: Values are the means ± standard deviations of triplicate measurements

There was a positive correlation ($r=0.887$ for G12 strain, $r=0.986$ for B3 strains, $r=0.959$ for W22 strain) between the EPS production at highest sugar concentration in the medium and optical density and the correlation was significant at the 0.05 (for G12) or 0.01 (for B3, W22) level. It has been shown with *L. casei* (Cerning et al., 1994) and *L. rhamnosus* (Gamar et al., 1997) that the presence of excess sugar in the medium (at concentrations between 10 and 20 g/L) had a stimulating effect on EPS production, although the growth was apparently reduced. The all strains in this study did not show this effect. For B3, G12 and W22, the optimal glucose concentration for both growth and EPS production was 30 g/L.

Gamar et al. (1997) reported that EPS production and yield were influenced by the carbon source and concentration. When *L. rhamnosus* was grown in a chemically defined medium on mannose 4%

w/v or glucose+fructose 2% w/v (ratio 1:1), the EPS production increased by three or four times whereas the final biomass concentrations were identical.

In conclusion, *L. delbrueckii* subsp. *bulgaricus* B3, G12 and *S. thermophilus* W22 were able to produce the EPS in the presence of various sugars; however, the most efficient carbon source was glucose. Furthermore, the sugar concentration had a marked effect on the EPS production. For instance, raising the glucose concentration resulted in increased EPS production.

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